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Contraceptive efficacy and antioxidant potential of *Leptadenia reticulata* bark extracts in male albino rats

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Contraceptive

Leptadenia reticulata (jivanti)

Testosterone

Spermatozoa

ABSTRACT

Birth control measures available are primarily for women which are hormonal supplements that are increasing cancer risks and reproductive health issues. Male contraceptive options are effective and available, i.e. barrier methods and vasectomy. Condoms are failure-prone and single-use, while a vasectomy is a permanent sterilization method done surgically, and reversion is not always successful and expensive. A promising oral male contraceptive drug candidate is yet to be discovered. This study investigated the contraceptive efficacy and antioxidant potential of various extracts of *Leptadenia reticulata* bark in male rats. To study the effects of various extracts (ethanolic and petroleum ether) of *L. reticulata* bark in male rats, oral administration at the dose level of 250 mg/kg body weight/ day was done for 60 days. Observations were made for body and organ weight, hematology, serum biochemical chemistry, testosterone and antioxidants, lipid profile, sperm parameters (density and motility) and histological changes (reproductive organs). As compared to control in treated groups (TP and bark petroleum ether extract), a significant reduction ($P \leq 0.001$) was perceived in sperm motility and density, as well as reproductive organ weight, serum testosterone, and serum antioxidant parameters like SOD. Histological observations revealed arrest in spermatogenesis and reduced seminiferous tubule diameter, mature Leydig cells, secondary spermatogonia, and spermatids which caused a substantial increase in LPO and GSH. From the research findings, it can be concluded that bark petroleum ether extract of *L. reticulata* possesses contraceptive potential in male albino rats and can serve as a safe and reversible oral contraceptive for males.

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1 Introduction

The present hunt or pursuit for safe oral contraception for the male is continued since ancient times (Jain et al. 2013). In family planning programs, the first choice is female contraceptive measures which are harmful. Furthermore, all of the methods tested to use for males as spermicides entering the cervix or infertility inducers, were also ineffective and unsafe. As a result, several scientists are evaluating alternative and complementary medicine to lower these adverse effects (Hifnawy et al. 2021). Male contraceptives are relatively less used and are few as compared to females. Primary contraceptive methods are classified into two groups, i.e. Traditional and Modern. Traditional male contraceptive methods include withdrawal and periodic abstinence. Modern techniques involve sterilization (vasectomy) and barrier methods (condoms). These methods account for 8.9% (United Nations, World Contraceptive Use 2009). Till date in market not a single oral male contraceptive is brought, despite of continuous efforts and experimental trials are done to introduce pharmacological agents and hormonal chemicals.

Due to side effects, irreversibility and incomplete efficacy of the results in most cases total arrest in spermatogenesis was observed (Kogan and Wald 2014). Present contraceptive methods for males have numerous adverse effects and unwanted pregnancies, which are rising at an unacceptable rate (Montaserti et al. 2007; Mishra et al. 2009). Many chemicals used in agriculture, such as pesticides, insecticides, herbicides and fertilizers, negatively affect fertility. They tend to have total arrest in the spermatogenesis process which is irreversible (El-Kashoury et al. 2009). Male contraception due to these adverse effects remains unacceptable worldwide (Beckman and Harvey 1996; Moore et al. 1996). Thus, the challenge is to search for a safe, reversible, and effective male contraceptive drug with the least negative impact. Due to the great benefit of health, plants and folk medicines have consistently been acclaimed. Nowadays, plants are safe sources of medications. Many plant extracts have been accessed concerning both the male and female antifertility potential. Various plant extracts exert different effects like reduction in sperm counts, altering mobility of sperm and bringing spermicidal reaction (Singh and Singh 2009). Some others can produce changes in hormonal levels, and some can make changes in the testis (Reddy et al. 1997). Many plant metabolites such as saponins, phenolic acids, steroids, flavonoids and alkaloids showed antifertility activity (Siddiqui et al. 1978; Chakravarty et al. 2003; Russo and Borrelli 2005; Manthri et al. 2011). Developing fertility control methods for males can achieve immense social and health benefits. Worldwide numerous medicinal plants with antifertility activities are reported. Still, hardly any contraception has been processed from extracts of plants, which is due to an inaccurate determination of their activities, and insufficient knowledge of the extract's active fraction and mode of action (Ghosh et al. 2002).

Leptadenia reticulata belonging to the family Apocynaceae, is one of the desert plants which might have antifertility potential but has still not been evaluated. Species of *Leptadenia* are mostly economically valued as they have therapeutic properties. According to Sivarajan and Balachandran (1994), this plant possesses lactogenic, rejuvenating and revitalizing properties. Chemical components reported in *L. reticulata* include terpenoids, phenolics, flavonoids, and esters. Qualitative tests revealed that the aerial part of the plant has terpenoids, alkaloids, sterols, tannin, saponins, flavonoids, carbohydrates and glycosides (Verma and Agarwal 1962; Pal et al. 2012; Hewageegana et al. 2014). *L. reticulata* has been reported to have Antianaphylactic, Antiasthamatic, Antimicrobial, Antioxidant, Anti proliferative, Hepatoprotective Potential, Anticancerous, Anti-Implantation, Antidepressant, Antiulcer, Antimalarial, Antiabortifacient, Anti-Implantation, Aphrodiasic and Anti-Inflammatory activities (Mohanty et al. 2017). It has been claimed that this plant has anti-implantation and anti-abortifacient properties in females. However, despite its use in herbal remedies for boosting fertility with other herbal plants, which lacks scientific support, its antifertility activity for males is yet to be evaluated. This study aimed to determine the antifertility capacity of *L. reticulata* bark extract, which might help find novel male oral contraceptives.

2 Materials and Methods

2.1 Collection of plant sample, identification and authentication

Bark of *L. reticulata* was collected from AFRI (Arid Forest Research Institute), Jodhpur, Rajasthan, India and was identified and verified by an expert at the Botanical Survey of India, Jodhpur with authentication number BSI/ AZRC/ I.12012/ Tech./ 2021-22 (PI-Id.). Samples were preserved in the BSI department for future reference.

2.2 Extract preparation

The bark of *L. reticulata* was air-dried in the shade to reduce the moisture content. Dried bark was finely ground to powder using a grinder, and 200 g of bark powder was mixed with 800 ml of ethanol (99.9%) and petroleum ether (40-60%) each. These mixtures were subjected to soxhlet extraction apparatus separately for 32 hours. To avoid sticking material in the flask bottom intermittent shaking was done. After 32 hours, the solution was filtrated using a muslin cloth, and the obtained filtrate was subjected to evaporation under reduced pressure to get a semi-solid paste of extracts. The extracted materials were weighed and stored at -4°C in sterile, airtight containers. These extracts were used to treat male albino rats. To prepare extracts for phytochemical assessment, sheets of Whatman No. 1 filter paper or extraction thimbles were used. The extraction apparatus' sample tube was inserted with a thimble filled with the bark powder. The bottom flask of the apparatus was filled with the solvent, and the unit was

then operated at the solvent's boiling temperature. The device was run until colourless solvent entered the syphon tube. To obtain the crude extract, the solvent was evaporated at reduced pressure (Sasikala and Kannikaparameswari 2023).

2.3 Phytochemical screening

Freshly prepared ethanolic and petroleum ether bark extract samples were sent to CDRI, Lucknow, for quantitative analytical analyses to identify various phytochemical components.

2.4 Maintenance of experimental animals

For the current investigation, albino rats, both male and female, weighing 150 - 200 g, were used. The experimental animals were kept in standard temperature conditions of 23±2 ℃ with 12-hour cycles of light and darkness. Before the onset of experiments, the animals were acclimated for seven days. For this, these experimental animals were kept inside animal houses in sanitized polypropylene cages that contained water bottles. Standard pellets and unrestricted access to water were available as a base diet. The Institutional ethical committee (IAEC) Reg. No. JNVU/IAEC/2020/03 approved all the experimental work conducted for this research, and guidelines from CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), The Government of India, were followed for animal handling. The veterinary advisor regularly supervised animals.

2.5 Physiological dose determination

Using the fixed-dose method described by Walum (1998), the LD50 (Lethal dose) was calculated. The dosage given was 250 mg/kg body weight for 60 days. Different bark extracts of *L. reticulata* were given by an oral route in the morning before 11 A.M. every day, and at the dose level of 0.01 mg/day, intramuscular injections of TP were given for 30 days to male albino rats.

2.6 Fertility test of male albino rats

Before administering extracts, rats were subjected to a fertility test that involved pairing up healthy male and female rats in a 3:1 ratio in individual cages for 5–6 days, i.e., 1 adult male with 3 adult females in a cage. Every female underwent a vaginal smear test for 5–6 days to check for the presence of sperm. Fertile males were identified by this test and used for this experiment.

2.7 Treatment protocol

Fertile and healthy albino male rats weighing 150 - 200 grams were used as model organisms. Five groups of animals were made; each group had five animals in duplicate. Group I (Control): Animals were fed a regular diet and given 2ml distilled water/day for 30 days. Group II: Testosterone propionate (TP) at 0.01 mg/day intramuscularly was given for 30 days. Group III: *L. reticulata* ethanolic extract of bark was orally administered to male albino rats at a dose level of 250 mg/Kg body weight/day dissolved in 2ml distilled for 60 days. Group IV: Petroleum ether extract of *L. reticulata* bark was orally administered for 60 days at the dose level of 250 mg/Kg body weight/day dissolved in 2ml distilled water to male albino rats. Group V: Petroleum Ether extract of bark was orally given at the dose level of 250 mg/Kg body weight/day dissolved in 2ml distilled water for 30 days and intra-muscular injections of TP at a dose level of 0.01 mg/day for 30 days were given to male albino rats. A standard diet was fed in all experimental groups throughout the experimental period.

2.8 Scheduling autopsy

Overnight fasted animals under mild anaesthesia were autopsied after completion of the experiment on the 30^{th} and 60^{th} day of treatments. Blood samples were collected using a clean and dry syringe via puncturing left ventricle and kept in both regular and EDTA-coated test tubes for the haematological, serum biochemistry, lipid profile, and antioxidant studies. For the separation of serum, centrifugation of blood for 15 minutes was done at 3000 rpm. For histological examination, all the vital organs (heart, kidney and liver) and reproductive organs (testes, cauda, caput, ventral prostate, vas deference and seminal vesicle) were dissected out, normal saline was used to clean the dissected organs, and 10% formalin was used to fix the tissues. It was further processed for histological slide preparations.

2.9 Body and reproductive organ weight determination

The initial and final body weight was weighed and recorded for all experimental animals. All vital and reproductive organs' weights were recorded after dissecting organs, removing extra tissues adhered to organs and cleaning them with saline.

2.10 Sperm motility and density

For this study, testes and epididymides were taken. After blood collection, epididymides immediately was separated, and cauda was taken for sperm motility and density (Prasad et al.1972).

2.11 Serum biochemistry

Serum total protein, globulin, albumin, urea, uric acid, creatinine, bilirubin and liver marker enzymes like SGOT (serum glutamic oxaloacetic transaminase), SGPT (serum glutamic pyruvic transaminase) and ALP (alkaline phosphatase) were estimated by using standard commercial kits.

2.12 Hematological parameters

Hematological parameters such as HB (Hemoglobin), HCT (hematocrit), TRBC (total red blood cells), MCV (mean corpuscular volume), MCHC (mean corpuscular hemoglobin concentration), PLT (platelet (thrombocyte) count), TLC (total leukocyltes count), PCT (procalcitonin test), MPV (mean platelet volume), RDW (red cell distribution width) and PDW (platelet distribution width) of blood samples were determined by using standard kits.

2.13 Lipid profile

Standard commercial kits were used for total serum cholesterol, VLDL (Very-low-density lipoprotein), HDL (high-density lipoprotein), LDL (low-density lipoprotein) and triglyceride estimation

2.14 Estimation of hormonal level

The Enzyme Immuno Assay method (EIA) was used for the determination of serum testosterone levels by using a commercial kit available.

2.15 Organ Histopathalogy

The vital (liver, kidney, heart) and reproductive organs (testes, cauda, caput, ventral prostate, vas deference and seminal vesicle), after fixation in 10% formalin, were dehydrated in successive grades of alcohol and then rinsed with xylene. Molten paraffin wax was used for embedding tissues. Microtome was used for cutting sections at a thickness of 5 µm, and for staining sections, hematoxylin and eosin were used. The slides were observed under a light microscope at 200 and 400 X magnifications for histopathological changes, especially in the reproductive organs. ImageJ software was used for testicular cell population counting or histometery of histological slides with the help of protocol given by Abercrombie (1946) and Dixon and Massey (1957).

2.16 Estimation of Antioxidant

2.16.1 SOD (Superoxide Dismutase)

SOD assay was carried out by the pyrogallol autoxidation protocol described by Marklund and Marklund (1974).

2.16.2 Catalase

In the presence of Catalase, the decomposition of H_2O_2 was estimated by Aebi (1984).

2.16.3 LPO (Lipid Peroxidation)

LPO was carried out following the procedure outlined by Ohkawa et al. (1979).

2.16.4 FRAP (ferric reducing antioxidant power assay or the ferric reducing ability of plasma)

FRAP assay was executed using a modified approach of Benzie and Strain (1996).

2.16.5 GSH (Glutathione)

GSH levels in serum were estimated based on the method given by Beutler et al. (1963).

2.17 Statistical analysis

One-way analysis of variance (ANOVA) was used to assess the data, after which Tukey's multiple comparison tests were conducted. Data analysis was done statistically using a graph pad prism.

3 Results and Discussions

The challenge is to search for a safe, effective, reversible oral contraceptive for the male. Humans use plants to improve health because of their lengthy folk uses, ensuring their safe use. Also, plant-based products are readily assessable, cost-friendly, and have negligible side effects. Recently, plant research for their medicinal use and properties, i.e., ethnobotanical information, has gained attention (Heinrich 2000). The present study assessed the antifertility activity of *L. reticulata* bark extract in male albino rats.

3.1 Extraction

Soxhlet extraction was carried out using ethanol and petroleum ether as solvents, and the percentage extraction yield for bark was 2.33% in ethanolic and 2.02% in petroleum ether. *Boerhaavia diffusa* and *Achyranthes aspera* root powder was used for the soxhelation extraction procedure with different solvents by Sasikala and Kannikaparameswari (2023) and recorded a 24 % yield from the *A. aspera* root ethanolic extract while in case of petroleum ether extract, it was recorded 17 %. Further, in the case of *B. diffusa, the* yield of ethanolic root extract was recorded at 26 %, while it was recorded at 13% in the case of petroleum ether extract. This yield is relatively high than the yield of *L. reticulata* bark extract.

3.2 Phytochemical screening

Bark extracts disclosed the presence of carbohydrates, starch, glycosides, protein, phytosterols, flavonoids, terpenoids, tannins, phenolic compounds and alkaloids. Three species of the family Asclepiadaceae (*Peruglaria tomentosa* L., *Pentatropis spiralis* (Forsk.) and *Calotropis procera* L.) were examined for phytochemistry from their crude extracts. Glycosides, alkaloids, saponins, tannins, terpenoids and flavonoids were found in the extracts after the phytochemical screening (Al-Dalahmeh et al.

2022). These constituents are quite similar to the reports of the present study.

3.3 Effect on body weight and reproductive organs weight

Rats administered with *L. reticulata* bark extract showed no noticeable changes in body weights. However, a noticeable reduction ($P \leq 0.05$) was noticed in the weight of the treated group's reproductive organs, such as testes, seminal vesicles, and epididymides, in comparison to the control (Table 1). A highly significant reduction was observed in groups II and IV. Results agree with the findings of Sharma et al. (2022) those, who recorded the antispermatogenic activity of *Momordica dioica* methanolic root extract. The weight of the testes significantly decreased after oral administration of the *M. dioica* methanolic root extract at a dose level of 50 mg/kg body weight/day ($p < 0.05$), although there was no discernible alteration in body weight or epididymis weight.

3.4 Effect on serum biochemistry and hematological parameters

Hematology and biochemical indicators showed no difference between the treated and the control groups after the oral administration of *L. reticulata* bark extracts. All results were found in the normal range. The non-toxic effect of the orally administered extract on the body's general metabolism is reflected by non-significant changes in serum biochemistry and haematology (Sripriya et al. 2011).

3.5 Effect on lipid profile

The orally fed bark extract of *L. reticulata* caused a marginal increase in lipid profile components like LDL, VLDL, HDL triglyceride and total cholesterol of control and treated groups. However, the increase remains statically insignificant in the amount of these components. Impairing in spermatogenesis results due to low androgen concentration. Increased cholesterol levels interfere with steroidogenesis in the testes (Agarwal et al. 2009). Steroidogenesis is affected by the increased cholesterol, while the increase observed was marginal, which may not have negatively impacted the androgen synthesis.

3.6 Effect on sperm motility and density

L. reticulata bark extract-fed rats showed decreased sperm parameters (testes and epididymides). Highly convincing ($P \leq 0.001$) falls in sperm motility and density (Table 2) were observed in group II and group IV as compared to the control group, and a significant increase in group V as compared to group II. It is a general fact that the fertilization capability of sperm is influenced by sperm motility (Amelar et al. 1980). A significant reduction in sperm motility and density of *L. reticulata* bark extract treated

Table 1 Body and Reproductive organ weight of various Bark extracts of *Leptadenia reticulata* treated male albino rats

TREATMENT GROUPS	Body Weight (gm)		Reproductive Organ Weight (gm/100gm Body Weight)			
	Initial	Final	Testes	Seminal Vesicle	Epidydimis	Ventral prostate
Group I (Intact Control)	195.74±3.45	200.03±1.87	1198.36±4.95	698.32±3.24	480.18±4.03	256.83±6.98
Group II (TP)	163.91±5.57	186.13±3.42	828.24±2.49^{c}	460.57±7.29^{c}	372.24±2.83^{c}	169.86±2.19^{c}
Group-III (Bark Ethanolic)	197.66±11.15	207±7.93	1051.39±44.30^{b,g}	557.43±37.50^{c,f}	430.01±33.50^{a,e}	206.92±16.23^{b,f}
Group-IV (Bark Petroleum Ether)	176±8.54	196.66±6.11	811.14±19.24^{c,h}	410.87±39.52^{c,e}	360.08±31.46^{c,h}	165.15±9.64^{c,h}
Group-V (Bark Petroleum Ether + TP)	224.48±11.68	244.92±13.97	990.10±72.99^{b,f}	422.84±28.91^{c,e}	460.09±20.53^{d,g}	238.20±20.70^{d,f}

Gr. II to IV compared with Gr. I: $P \leq 0.05$= a, $P \leq 0.01$= b, $P \leq 0.001$ = c, Non-significant = d; Gr. III & IV compared with Gr. II: $P \leq 0.05$= e, $P \leq 0.01$ = f, $P \leq 0.001$= g Non- significant = h

Table 2 Sperm dynamics of various Bark extracts of *Leptadenia reticulata* treated male albino rats

TREATMENT GROUPS	SPERM MOTILITY (%)	SPERM DENSITY	
		CAUDA (million/ml)	TESTES (million/ml)
Group I (Intact Control)	86.06±3.24	68.01±0.97	4.6±0.32
Group II (TP)	15.12±0.91^{c}	14.72±1.41^{c}	1.16±0.30^{c}
Group-III (Bark Ethanolic)	79.35±1.62^{a,g}	42.13±2.50^{ca,g}	4.83±0.67^{d,g}
Group-IV (Bark Petroleum Ether)	10.32±0.99^{c,h}	4.44±0.40^{c,g}	0.78±0.02^{c,h}
Group-V (Bark Petroleum Ether + TP)	68.68±3.22^{b,g}	54.46±4.82^{b,g}	3.30±0.43^{a,g}

Gr. II to IV compared with Gr. I: $P \leq 0.05$= a, $P \leq 0.01$= b, $P \leq 0.001$ = c, Non-significant = d; Gr. III & IV compared with Gr. II: $P \leq 0.05$= e, $P \leq 0.01$ = f, $P \leq 0.001$= g Non- significant = h

Table 3 Histometerical Parameters of Testes and Epididymides of various Bark extracts of *Leptadenia reticulata* treated male albino rats

TREATMENT GROUPS	Seminiferous Tubule Diameter (µm)	Epithelial Cell Height (µm)	
		Caput	Cauda
Group I (Intact Control)	260.93±10.31	39.20±3.64	29.58±0.61
Group II (TP)	150.45±4.93^{c}	30.34±0.88^{a}	21.03±1.48^{c}
Group-III (Bark Ethanolic)	206.59±16.64^{b,f}	33.93±3.32^{d,h}	27.48±2.08^{d,e}
Group-IV (Bark Petroleum Ether)	144.96±8.37^{c,h}	26.39±2.44^{b,e}	19.93±1.56^{c,h}
Group-V (Bark Petroleum Ether + TP)	245.50±16.94^{d,g}	30.85±2.21^{a,h}	23.90±1.84^{b,h}

Gr. II to IV compared with Gr. I: P ≤ 0.05= a, P≤ 0.01= b, P≤ 0.001 = c, Non-significant = d; Gr. III & IV compared with Gr. II: P ≤ 0.05= e, P ≤ 0.01 = f, P ≤ 0.001= g Non- significant = h

groups was observed. A highly significant decrease was observed in bark petroleum ether extract, and reversibility of this reduction was observed in the combinational treatment of this extract with TP (Testosterone propionate). Reduction in male rat sperm motility after the extract administration may be due to the reduction in ATPase and succinate dehydrogenase levels (Rao 1987). Or it may be due to increased membrane fluidity and destruction of sperm membrane by lipid peroxidation, which results in cell apoptosis by damaging DNA by inactivating the membrane channels, proteins and enzymes (Oborna et al. 2009).

3.7 Effect on histopathology

After treatment with *L. reticulata* bark extracts, the main effect on histology was observed in reproductive organs. Seminiferous tubules were observed with degenerative changes such as arrest in spermatogenesis, highly convincing (P ≤ 0.001) reduction in seminiferous tubule diameter and a denoting reduction in the secretion of ventral prostate and seminal vesicle (Table 3). The reduction was highly significant (P ≤ 0.001) in correlation to control, especially in groups II and IV, while a notable increase in

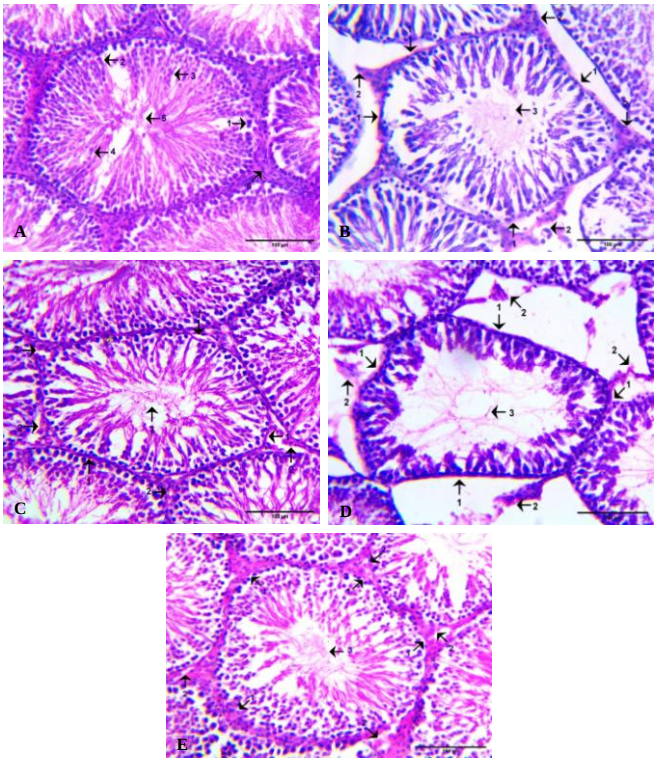

Figure 1 Microphotographs of different groups Testes; A. Control male albino rat Testes labeling showing various spermatogenesis stages used for histometery 1. Spermatogonia; 2. Primary Spermatocyte; 3. Secondary Spermatocyte; 4. Spermatid; 5. Lumen filled with spermatozoa; 6. Leydig Cells; HE, 200× B; TP- Testosterone propionate, C. Bark Ethanolic and D. Bark Petroleum Ether Extract treated male albino rat testes labeling showing: 1. Reduction in diameter of seminiferous tubule; 2. Reduction in ledyig cells; 3. Leumen with reduced spermatozoa; HE, 200×. E. TP + Bark Petroleum Ether Extract treated male albino rat Testes labeling showing 1. Recovering diameter of seminiferous tubule; 2. Regenerating ledyig cells; 3. Leumen with increased spermatozoa (HE, 200 x)

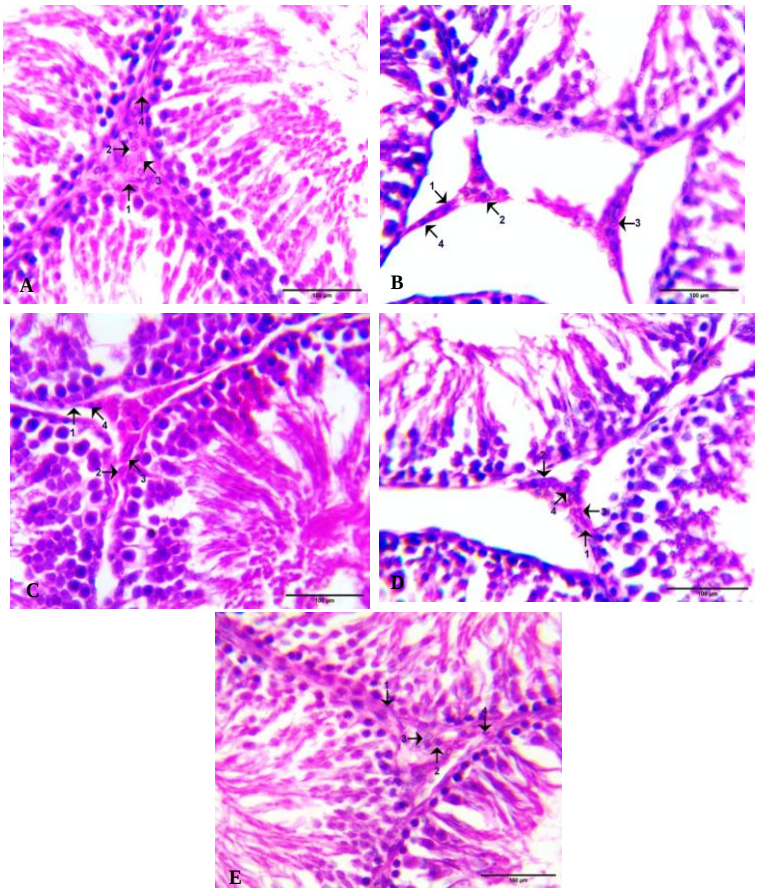

Figure 2 Microphotographs of interstitial cells of different groups: A. Control (Group I), B. TP-Testosterone propionate (Group II), C. Bark Ethanolic Extract (Group III), D. Bark Petroleum Ether Extract (Group IV) & E. TP + Bark Petroleum Ether Extract (Group V) labeling showing: 1. Fibroblast Cells; 2. Immature Ledyig Cells; 3. Mature Ledyig Cells; 4. Degenerating Cells (HE, 400×)

these parameters of group V as compared to group II was observed (Figures 1 and 2). In the present investigation, orally administered bark extract of *L. reticulata* reduced the weight of reproductive organs. Reduction in testes and seminiferous tubule diameter reflects damage or degenerative changes in these organs. In assessing spermatogenesis, the first way is to assess the testicular size because approximately 98% of testis mass comprises tubules and germinal cells (Keel and Abney 1980). Degenerative changes in the testis, epididymis, vas deferens and disintegration of Leydig cells reflect anti-androgenic activity (Rajan et al. 2013).

3.8 Effect on testicular cell population

The bark extract of *L. reticulata* treatment significantly ($P \leq 0.001$) reduced the secondary spermatogonia, spermatids, and mature Leydig cells and significantly ($P \leq 0.001$) increased in degenerative cells (Table 4). Compared to the control, the reduction in groups II and IV was highly significant, while a significant increase was noticed in these parameters in group V (Figures 1 and 2). The diameter of the seminiferous tubule, testicular and Leydig cell population dynamics were significantly reduced by *L. reticulata* bark extract. Compared to an ethanolic extract of bark, reduction in testicular and Leydig cell population was more significant in petroleum ether extract of bark. The reduction was more prominent in the secondary spermatocytes, spermatids and mature leydig cells. Reduction in diameter and surface area of the seminiferous tubule is attributed to steroids (De Souza et al. 2017). Atrophy of seminiferous tubules might be caused due to reduction in FSH and testosterone levels, which is a causative agent in surface area and the reduction of seminiferous tubule diameter. The number of germ cells in the testes is affected by spermatogenesis disruption. Increased lumen surface area and reduction in spermatogenic cells in seminiferous tubules lead to morphological changes (Yama et al. 2011). The primary source for androgens or steroidogenesis is leydig cells in the interstitial area of seminiferous tubules (Shima et al. 2013). Degenerative changes in leydig cells may be due to a decline in LH secretion (Nair et al. 1995).

Table 4 Testicular Cell Population Dynamics of various Bark extracts of *Leptadenia reticulata* treated male albino rats

TREATMENT GROUPS	Germinal Cell Type				Interstitial Cell Type			
	Spermatogonia	Primary Spermatocytes	Secondary Spermatocytes	Spermatids	Fibroblast Cells	Immature Leydig Cells	Mature Leydig Cells	Degenerating Cells
Group I (Intact Control)	46.19±1.52	35.21±3.51	86.67±4.50	148.26±2.15	60.65±3.21	50.43±1.52	72.36±2.51	17.29±1.52
Group II (TP)	36.36±2.51^{b}	33.28±1.52^{d}	41.38±3.51^{c}	29.97±2.51^{c}	54.66±3.05^{d}	40.53±4.50^{a}	41.71±1.52^{c}	64.47±2.51^{c}
Group-III (Bark Ethanolic)	41.92±2.32^{a,e}	29.52±0.84^{a,h}	78.77±1.75^{d,g}	138.86±4.16^{a,g}	62.85±3.33^{d,e}	46.28±1.49^{a,h}	59.67±3.25^{b,g}	32.14±2.41^{c,g}
Group-IV (Bark Petroleum Ether)	32.93±1.70 ^{c,h}	29.93±2.14^{a,h}	38.91±0.46^{c,h}	25.98±1.49^{c,h}	55.5±2.42^{d,h}	43.16±1.83^{b,h}	39.8±3.54^{c,h}	61.83±3.31^{c,h}
Group-V (Bark Petroleum Ether + TP)	41.89±1.86^{a,e}	32.96±2.11^{d,h}	78.61±3.49^{d,g}	139.33±4.75^{a,g}	60.30±1.96^{d,e}	49.39±2.06^{d,e}	65.98±2.32^{d,g}	25.66±1.95^{c,g}

Gr. II to IV compared with Gr. I: P ≤ 0.05= a, P≤ 0.01= b, P≤ 0.001 = c, Non-significant = d; Gr. III & IV compared with Gr. II: P ≤ 0.05= e, P ≤ 0.01 = f, P ≤ 0.001= g Non- significant = h

3.9 Effect on serum testosterone level

Compared to the control, *L. reticulata* bark extract significantly (P 0.001) decreased serum testosterone levels in all treated groups (Figure 3). This reduction was highly significant in groups II and IV. However, group V showed a considerable increase as compared to group II. A primary and representative androgen that controls the progression of spermatogenesis is testosterone (Turner et al. 1984). Decreased testosterone levels may cause alteration in cell signalling, DNA repair, apoptosis, metabolism, RNA processing and meiosis; this might result from testosterone requirement in male germ cells development and maturation (Stanton et al. 2012). Significant reduction in serum testosterone was noticed in bark extract administered groups, possibly due to decreased mature leydig cells (Gupta et al. 2011), and the restoration of serum testosterone was observed in TP combined with the extract.

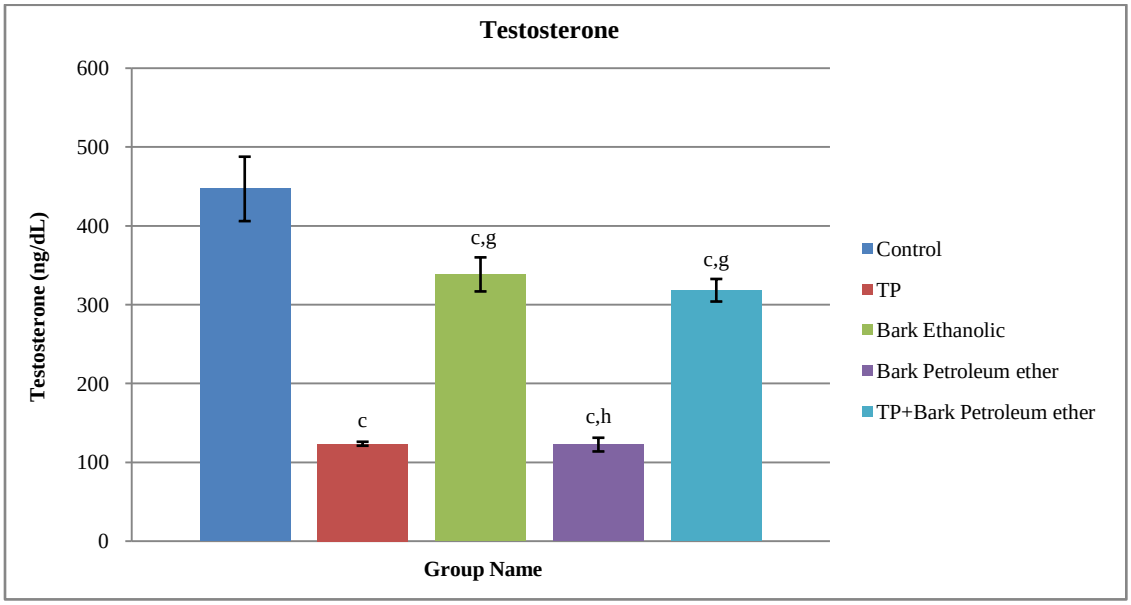

Figure 3 Effect on serum testosterone level in male albino rats after oral administration of bark extracts of *L. reticulata*. Data was expressed in Mean ± SD. Error bars are representing SD of Mean and superscripts denoting significance level.

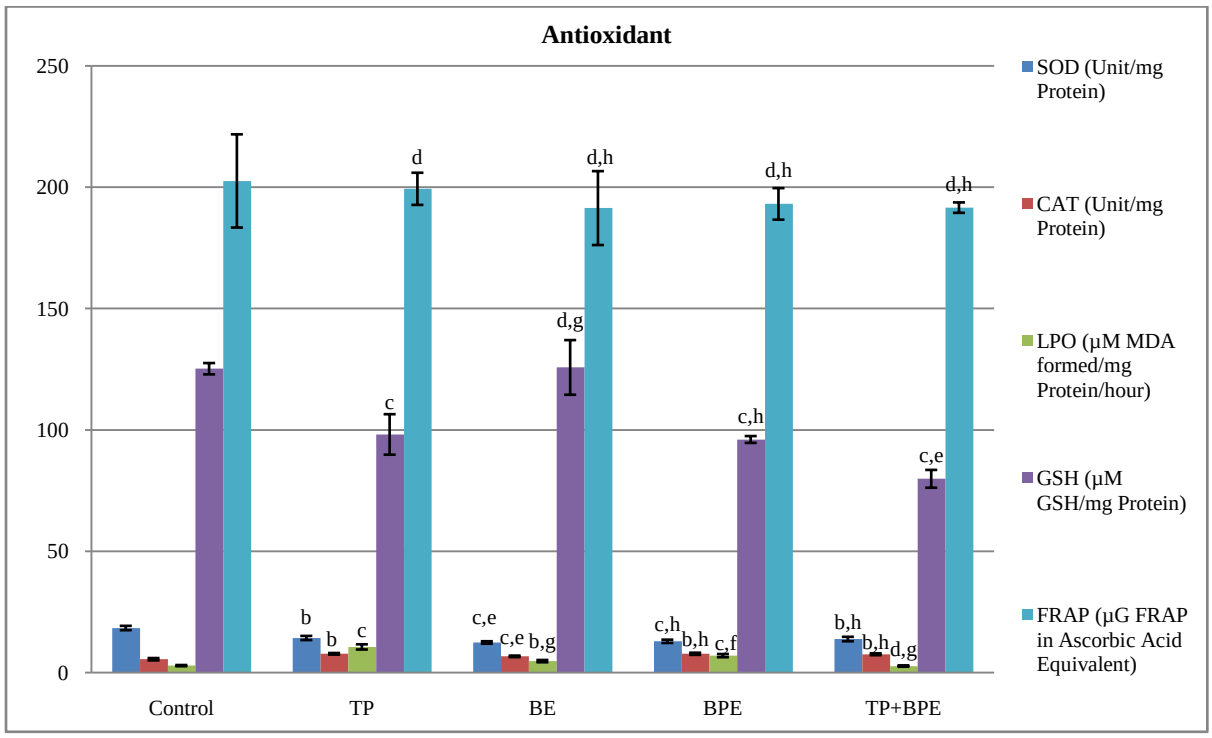

Figure 4 Effect on serum antioxidant assay in male albino rat after oral administration of *Leptadenia reticulata* bark extracts (Sod, Catalase, LPO, GSH and FRAP). Data was expressed in Mean ± SD. Error bars are representing SD of Mean and superscripts denoting significance level. TP- Testosterone Propionate, BE- Bark Ethanolic extract, BPE- Bark Petroleum Ether extract and TP+BPE- Testosterone Propionate + Bark Petroleum Ether extract.

3.10 Effect on serum antioxidant level

Like other parameters, *L. reticulata* bark extract treatment significantly reduced ($P \leq 0.001$) SOD and GSH and significantly increased Catalase ($P < 0.01$) and LPO concentration ($P \leq 0.001$) in all groups except group III which resulted in no significant change as compared to control. While the FRAP assay produced negligible differences from the control (Figure 4). Low oxygen conditions in reproductive organs may scavenge the free radical-mediated damages as oxidative stress is one of the barriers in leydig cell steroidogenesis and spermatogenesis (Smith et al. 2007). Prevention of lipid peroxidation of plasma membrane involves Catalase, GSH and superoxide dismutase (SOD), which is involved in scavenging superoxide ions (Jeulin et al. 1989) and converts them into (H_2O_2) and molecular oxygen (O_2), which in turn are converted to H_2O and O_2 Catalase and GSH. These help in maintaining decreased levels of LPO.

Increased serum LPO levels were observed after the administration of *Cynoglossum zeylanicum* extracts. This may be due to a decreased antioxidant potential of extract as it has reduced SOD, Catalase and GSH levels (Anitha et al. 2013). In contrast, the present findings showed a significant decline in SOD but a marginal rise in Catalase, a significant decline in GSH, and an elevation in the serum status of LPO. Additionally, non-significant change in the FRAP assay was seen in the treated groups of bark extracts, and serum antioxidant activity was restored in the treated group that had a combination of TP and bark petroleum ether extract. The increased levels of SOD, and Catalase, significant decreases in LPO and GSH, and non-significant changes in FRAP demonstrate the reversibility of the extract's effect on fertility. SOD, Catalase and GSH levels increase in serum, maintain antioxidant potential and decrease reactive oxygen species, which is reflected in LPO levels (Mruk et al. 2002). This contraceptive potential of bark extract is possible because of the different flavonoids, saponins and phenolic compounds in the extract.

It may be inferred that *L. reticulata* bark petroleum ether extract has an antifertility effect, most likely due to the degeneration of leydig cells responsible for maintaining testosterone levels in the blood. A possible mechanism for this contraceptive potential can be concluded that the drug (Extract) might have either blocked the maturation of leyding cells, i.e., the pathway of leydig cell development from immature leydig cell to mature leydig cell or the oxidative stress may have interfered with the leydig cell to produce testosterone level. Low testosterone level in the blood has affected the sertoli cells and spermatogenesis arrest, or the extract might have disturbed the testosterone synthesis pathway. Further investigation regarding *in silico* targeting the receptor and enzyme involved in testosterone synthesis is underway.

Conclusion

Bark petroleum ether extract of *L. reticulata* has significantly reduced the sperm motility, density, and testicular cell population dynamics, especially seminiferous tubule diameter, secondary spermatocytes, spermatids and mature leydig cells. Testosterone level was also significantly decreased after the extract administration. These parameters were recovered when the extract was provided in combination with TP, which shows reversibility of the extract's effect. It is obvious from the observations and results of the above research findings that the bark petroleum ether extract has contraceptive activity and can be used as a potent reversible herbal male oral contraceptive to regulate fertility without any side effects after extensive clinical trials.

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Conflict of Interest

No conflicts.

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